



## **Systems biology perspective for studying the gut microbiota in human physiology and liver diseases**

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## Review

## Systems biology perspective for studying the gut microbiota in human physiology and liver diseases

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## ABSTRACT

The advancement in high-throughput sequencing technologies and systems biology approaches have revolutionized our understanding of biological systems and opened a new path to investigate unacknowledged biological phenomena. In parallel, the field of human microbiome research has greatly evolved and the relative contribution of the gut microbiome to health and disease have been systematically explored. This review provides an overview of the network-based and translational systems biology-based studies focusing on the function and composition of gut microbiota. We also discussed the association between the gut microbiome and the overall human physiology, as well as hepatic diseases and other metabolic disorders.

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## Abbreviations

ALD	alcoholic liver disease;
BCAA	branched chain amino acids;
CCL	CC chemokine ligand;
cFBA	community Flux Balance Analysis;
CASINO	Community And Systems-level Interactive Optimization;
COMETS	Computation Of Microbial Ecosystems in Time and Space;
dFBA	dynamic Flux Balance Analysis;
DM	diabetes mellitus;
DMMM	Dynamic Multi-species Metabolic Modeling;
FLYCOF	Flexible sYnthetic Consortium Optimization;
MCM	Microbial Community Modeler FXR, farnesoid X receptor;
	GEM, genome-scale metabolic model;
HCC	hepatocellular carcinoma;
IL	interleukin;
MAPK	mitogen-activated protein kinase;
MCM	Microbial Community Modeler; NAFLD, non-alcoholic fatty liver disease;
NASH	non-alcoholic steatohepatitis;
NASH	non-alcoholic steatohepatitis;

NF-κB	nuclear factor-κB;
SCFA	short-chain fatty acids;
T2DM	type 2 diabetes mellitus;
TGR5	transmembrane G protein-coupled receptor 5 (also known as GPR109A);
TLR	toll-like receptor.

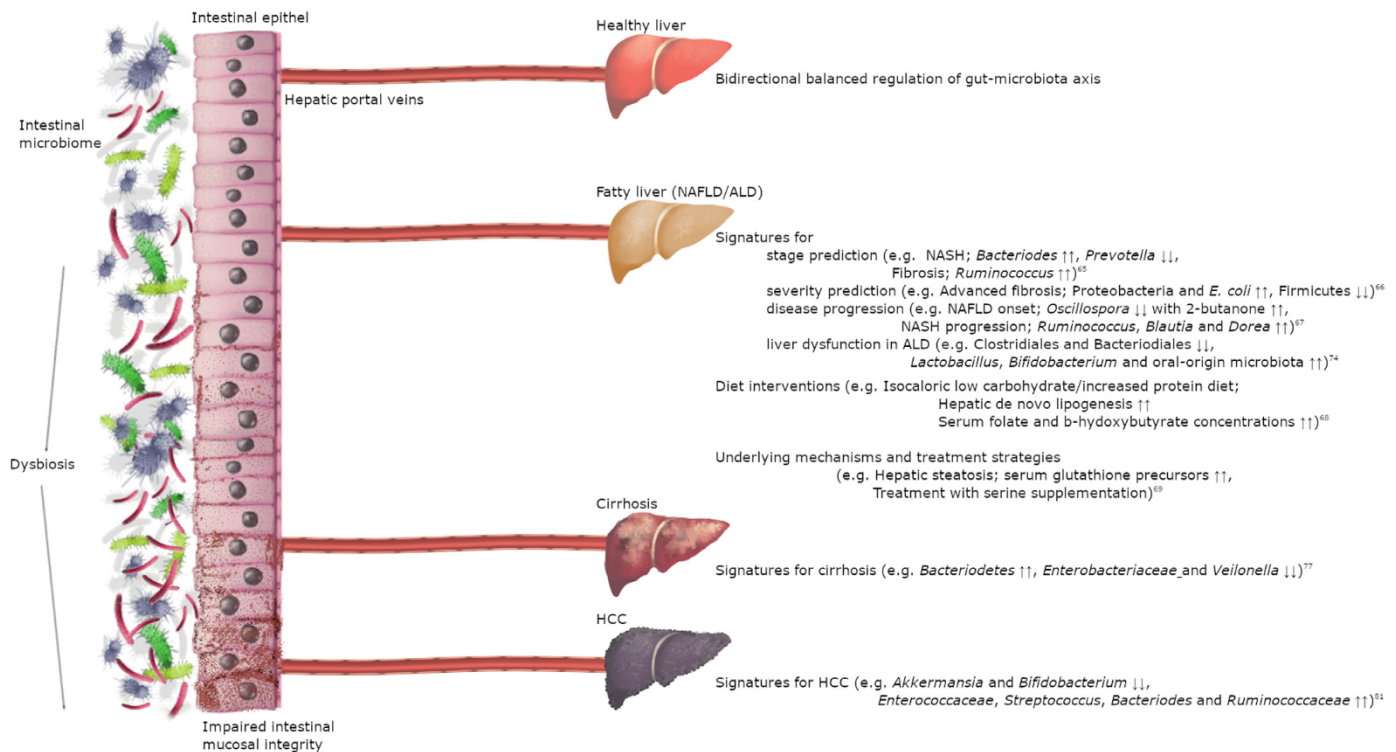
## 1. Introduction

The human gastrointestinal tract is inhabited by a complex microbial community comprising more than a trillion cells of approximately 1800 genera [1]. There is increasing evidence that this diverse microbial habitat has an important contribution to the metabolism of dietary components and overall regulation of health status. This has triggered a large amount of scientific interest into the investigation of microbiota and derived products, and the recognition of metabolic links, especially along the liver and gut bidirectional relationship [2,3].

The human host benefits from the metabolism of the microorganisms in the human gut, such as degradation of dietary indigestible carbohydrates and peptides which are consequently absorbed by the host and serve as an energy source. The main end products of bacterial metabolism are short-chain fatty acids (SCFAs, namely acetate, propionate, and butyrate),

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**Fig. 1.** Recent systems biology studies elucidate the close connection between gut microbiota and liver. Upper part of the figure reflects the healthy state of liver-gut axis. Alterations in the microbial composition and impaired intestinal barrier elucidated as pathogenic factors in various types of liver diseases by recent systems biology studies. (NAFLD/ALD, non-alcoholic fatty liver disease/alcoholic liver disease; NASH, non-alcoholic hepatosteatosis; HCC, hepatocellular cancer).

branched-chain amino acids (BCAAs, namely leucine, isoleucine, and valine), tryptophan-derived metabolites (mainly indoles and tryptamine) and trimethylamine, whose association with human physiology and various diseases have been increasingly shown by recent studies [4–6]. Furthermore, the microbiota is also involved in the regulation of bile acids, which in turn, can modulate glucose and lipid metabolism through FXR and TGR5 signaling [7]. Diet, xenobiotics, the intestinal environment and microbiota composition affect metabolism of microbiome-derived metabolites; thus, identifying their exact roles in human physiology is challenging [8].

The gut microbiota also plays a crucial role in the overall health of the body through the maintenance of intestinal mucosal integrity, the synthesis of essential amino acids and vitamins, the bioconversion of dietary complex molecules, the biotransformation of oral drugs, and the production of hormones and neurotransmitters [9–11]. An imbalanced gut microbiome, with the contribution of host genetic characteristics and environmental factors (e.g. diet, drugs), may lead to the development of a range of immune-mediated diseases and conditions including diabetes mellitus (DM), obesity and various types of liver diseases (Fig. 1).

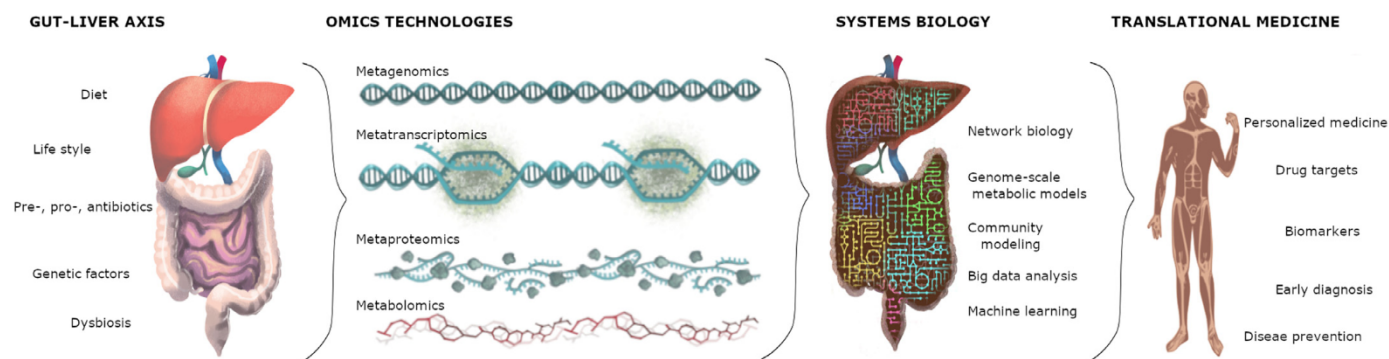
The composition, functions, and interactions of the intestinal microbiota have been systematically studied by using novel technologies to elucidate the underlying mechanisms that might account for the pathological processes, with the purpose of prevention, diagnosis, and treatment of diseases [12]. The ultimate goal of this new era is to achieve personalized medicine, which will provide the most compatible treatment for a specific patient by increasing the efficacy of treatments, whilst reducing the adverse effects and health expenses. Therefore, data integration and knowledge discovery capabilities of novel bioinformatics methodologies have emerged as game-changing tools for the discovery of biomarkers and drug targets, as well as the development of efficient treatment strategies such as diet interventions and fecal microbial transplantation. [13,14]

From this perspective, we reviewed state-of-the-art systems biology studies in the context of how environmental changes affect microbiota function and composition, and in turn how this is associated with human physiology and liver diseases. Here, we: (1) describe the synthetic gut microbe studies that have evaluated the bacterial composition and microbiota-derived metabolites under varying conditions in human intestine; (2) summarize the systems biology studies elucidating the role of altered gut microbiota on human metabolism in healthy and obese subjects and diabetic patients, as well as the role of microbiota on drug biotransformation; and (3) outline the novel studies that provide evidence for the interplay between microbiota and hepatic diseases, including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), liver cirrhosis, and hepatocellular carcinoma (HCC), and its possible diagnostic and therapeutic potential.

## 2. Systems biology approaches in understanding gut microbial communities

Advances in omics technologies have enabled gains in mechanistic insights of the human liver and gut microbiota in health and disease states. Omics technologies in microbiome studies are comprehensively described elsewhere [15], and are described briefly in Box 1. However, omics technologies, as a reductionist approach, have focused on describing complex biological systems in their simplest levels through concrete individual statements, but predicting the behavior of many interacting parts of systems has been highly improbable. Therefore, holistic approaches are necessary to make predictions for studying cellular and systemic functions (Fig. 2). The development and analysis of integrative biological networks can be used to make further predictions about the system-level properties through the use of genome-scale metabolic models (GEMs) [16]. GEMs are the stoichiometric reconstructions of the entire metabolism within a cell or tissue, which provide





**Fig. 2.** Interactions between human gut and liver have been deciphered by various omics technologies. Systems biology methodologies integrate high-throughput omics data to develop high-quality translational research and personalized medicine.

a link between genomic information and associated biochemical reactions [17]. GEMs have been presented and continuously refined to compile all gene–protein–reaction–metabolite associations with transport processes to simulate the complex relationship between the genotype and phenotype of an organism [18]. Systems biology-based studies that employ biological networks including GEMs, transcriptional regulatory, protein–protein interaction, signaling and co-expression networks in understanding the human liver physiology in healthy and diseased states have been extensively reviewed elsewhere [2].

Synthetic gut microbe networks decipher population interactions in multiple microbial species. Computational metabolic modeling of interspecies interactions predicts how members of the gut flora promote each other for growth and/or compete for space and nutrients [19]. Microbial relationships at different taxonomic levels have been simulated by using extended constraint-based metabolic flux analysis (e.g. OptCom, cFBA, CASINO, MMinte, SteadyCom) [20–24]. However, the dynamic equilibrium of the human microbiome challenges the methods that allow the prediction of interactions based on steady-state assumption. Similarly, the majority of flux prediction methods rely on a maximization of the biomass or ATP, but this is not the case for all members of the microbial community. Dynamic modeling frameworks (e.g. dFBA, DyMMM, D-optCom, COMETS, MCM, BacArena, FLYCOP) have been developed to achieve more realistic simulations of microbial ecosystems [25–31]. An overview of various community modeling frameworks is presented in Tables 1 and 2. A compilation of studies that illustrates environmental alterations on behaviors of gut microbial communities (with 4 or more species) is detailed in this review paper.

Bacteria in the human gut microbiome inhabit the same physical location and connect instantly with each other. The sharing of a common living space with all microbial partners is regulated via metabolic cross-feeding. Predictions of metabolite production levels within the representative communities of human gut microbiota members have rendered efficient estimations of the human gut microbiota. For instance, applying a spatial and temporal multi-scale modeling approach (namely BacArena) to a sample group of seven bacteria of the human gut showed the importance of the different location-based densities of mucus glycans in terms of niche formations and ultimately, the habitat topology on the whole. The approach also demonstrated the multi-member exchange of SCFAs as a contributing factor to the concentration values of community members, which were in line with previously published experimental findings [30].

High-level methodological examination of microbial metabolic exchanges allows further discovery of microbial metabolic relationship factors relating to ecological stability and vulnerability. For in-

stance, *in silico* interactions between *Escherichia* sp., *Akkermansia muciniphila*, *Subdoligranulum variable*, and *Intestinibacter bartlettii* and their extracellular environment were evaluated with the concept of synthetic lethality analysis based on the flux balance analysis to elucidate the possible effect of metformin treatment on gut microbiota composition in patients with type 2 diabetes mellitus. Authors observed that *Escherichia* sp. and *S. variable* were able to contribute to the production of short-chain fatty acids under aerobic and anaerobic conditions, and *Escherichia* sp. withstood most nutrient deficiency out of all species studied [32]. In another study, the growth interdependencies of *Desulfovibrio piger* between 8 other microbial species under distinct metabolic limitations (oxygen, chondroitin sulfate, and fructose) were evaluated using the MMinte framework. In this study, *D. piger* growth was dependent if *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Bacteroides caccae*, *Clostridium symbiosum* or *Escherichia coli* were under the absence of oxygen, but no dependence was observed if oxygen was available [23]. Similarly, a recent metabolic model-based approach of pairwise interactions of 11 gut bacteria suggested that cross-feeding behavior varied under different nutrient and atmospheric conditions of the gastrointestinal tract. For instance, the mutualistic interactions of *Lactobacillus plantarum* under anoxic conditions were abolished in the presence of oxygen [33]. Overall, these results may suggest that microbes rely on each other more when they are under poor nutrient and oxygen availability.

The commensal behavior of the intestinal microbes might be altered with the production and consumption of extracellular compounds under different diet conditions. The computational framework CASINO demonstrates the multifaceted anabolic and catabolic interactions amongst food intake, gut microbiota and host. The model, which accomplishes predictions in line with human values, reveals that various amino acid and SCFA levels, in addition to the microbiota composition, are impacted by microbial genome diversity and diet [22]. Likewise, metabolic flux arrangements in accordance with steady state boundaries can be estimated by using the SteadyCom optimization framework. Following an assembly of four *E. coli* double auxotrophic mutants, the framework was tested on a microbiota representation containing microbes from the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. With little need for constraints other than diet perturbation, SteadyCom accordingly generates predictions of abundance fluctuations, which are backed up by experimental gut microbiota representations [24].

Integration of omics data onto the metabolic models is a novel methodology for a definitive understanding of microbial functions and dynamics. A pioneer study from Shoaie et al., simulated the interactions between relevant representatives (*B. thetaiotaomicron*, *Eubacterium rectale* and *Methanobrevibacter smithii*) of the human gut



**Table 1**

Summary of community modeling frameworks (steady-state) using genome-scale metabolic models.

Name	Programming languages	Definition	Research organism	Availability	Reference
SteadyCom	MATLAB	Prediction of the flux distributions and maximum growth rate of a community (independent from number of organisms) in a time-averaged approach	1. <i>Bacteroides thetaiotaomicron</i> 2. <i>Eubacterium rectale</i> 3. <i>Faecalibacterium prausnitzii</i> 4. <i>Enterococcus faecalis</i> 5. <i>Lactobacillus casei</i> 6. <i>Streptococcus thermophilus</i> 7. <i>Bifidobacterium adolescentis</i> 8. <i>E. coli</i> 9. <i>Klebsiella pneumoniae</i>	<a href="https://github.com/maranasgroup/SteadyCom">https://github.com/maranasgroup/SteadyCom</a>	Chan et al. [24].
MMinte	Python	A compartment-based simulation of microbial interactions from an association network and assessment of 16S rDNA data	1. <i>Desulfovibrio piger</i> 2. <i>Bacteroides thetaiotaomicron</i> 3. <i>Bacteroides caccae</i> 4. <i>Bacteroides ovatus</i> 5. <i>Eubacterium rectale</i> 6. <i>Marvinbryantia formatexigens</i> 7. <i>Collinsella aerofaciens</i> 8. <i>E. coli</i> 9. <i>Clostridium symbiosum</i>	<a href="http://www.github.com/mendessoares/MMinte">www.github.com/mendessoares/MMinte</a>	Mendes-Soares et al. [23].
CASINO	MATLAB	An optimization algorithm which incorporates the systems-level topology with iterative organism-level and multi-level optimization to predict metabolic interactions within the microbial communities	1. <i>Bifidobacterium adolescentis</i> 2. <i>Bacteroides thetaiotaomicron</i> 3. <i>Eubacterium rectale</i> 4. <i>Faecalibacterium prausnitzii</i> 6. <i>Lactobacillus reuteri</i>	–	Shoaie et al. [22].
cFBA	Python	A methodology which predicts community metabolic activities at a balanced growth rate by using a simplified multi-objective optimization approach	<i>E. coli</i>	<a href="http://cbmpy.sourceforge.net/">http://cbmpy.sourceforge.net/</a>	Khandewal et al. [21].
optCom	UNIX/ LINUX	A pioneer multi-level and multi-objective optimization formulation to describe species- and community-level fitness analysis of microbial communities.	1. <i>Geobacter sulfurreducens</i> 2. <i>Clostridium cellulolyticum</i> 3. <i>Clostridium cellulolyticum</i> 4. <i>Methanococcus maripaludis</i>	–	Zomorodi et al. [20].

by using genome-scale metabolic models based on transcriptome data [34]. The models predicted that *B. thetaiotaomicron* produced more butyrate with *E. rectale* and more acetate with *M. smithii*, which resulted in more methane production of *M. smithii*. Predictions of the secreted SCFA profiles with *in silico* evaluations in different combinations of gut ecosystems were comparable with the experimental data of germ-free mice [34]. Nutrient cross-feeding between *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii* has also been explored by different groups and findings consistently indicate that SCFAs are modulated by environmental conditions, as well as microbial interactions [35,36].

### 3. Role of altered gut microbiota on human metabolism

To date, data from observational and comparative studies have highlighted that gut microbiota metabolism and the human liver are closely connected with each other via enterohepatic circulation, and the results of this bidirectional relation have been linked to overall health and disease [37–39]. The holistic approaches of systems biology have been gaining attention in medical management to promote health and prevent disease, which in turn renders possible the practice of predictive, preventive, personalized and participatory (P4) medicine [40]. Examples of systems biology studies highlighting the effect of environmental factors (e.g. diet, drugs) on the microbiota and the subsequent impact on host health are included in this section.

#### 3.1. Health and microbiota

Understanding the role of the microbiota on well-being and disease necessitates the use of data mining and integration approaches as part of the current comprehensive view towards systems biology. As a seminal example, Price et al. analyzed the personal and multi-omics longitudinal data of 108 individuals and employed a correlation network to define related markers of health and disease. The authors identified several relationships between specific taxa and metabolites, as well as negative correlation of microbiome  $\alpha$ -diversity with some immune response proteins [41]. In another study, statistical analysis of an early school-age cohort of Dutch children revealed that environmental factors (especially breastfeeding duration and dietary habits) influenced microbial composition with a significant enterotype association. High dietary fiber consumption and low plasma insulin levels were correlated with *Bacteroides* and *Prevotella* enterotypes, but not with *Bifidobacterium* enterotype, which has a lower microbial diversity and association with a shorter breastfeeding duration [42].

The essential role of diet in well-being or complex diseases has gained importance and landmark computational studies have evaluated the association of microbiota profiles with disease development [43,44]. Mardinoglu et al. reconstructed a generic mouse metabolic reaction GEM, together with 28 tissue-specific and 4 functional GEMs for the small intestine, colon, liver and white adipose tissues based on proteomics and transcriptomics data from conventionally raised and germ-free mice. The authors simulated

**Table 2**

Summary of community modeling frameworks (dynamic) using genome-scale metabolic models.

Name	Programming languages	Definition	Research organism	Availability	Reference
FLYCOP	Python	A novel spatiotemporal modeling approach to explore multiple consortium configurations through stochastic local search process	1. <i>E. coli</i> 2. <i>Synechococcus elongatus</i> 3. <i>Pseudomonas putida</i>	<a href="https://github.com/beatrizgj/FLYCOP">https://github.com/beatrizgj/FLYCOP</a>	Beatriz García-Jiménez et al. [31].
BacArena	R	A rule-based spatial and temporal multi-scale modeling approach which combines FBA with individual-based modeling	1. <i>Anaerostipes caccae</i> 2. <i>Bacteroides thetaiotaomicron</i> 3. <i>Blautia producta</i> 4. <i>E. coli</i> 5. <i>Clostridium ramosum</i> 6. <i>Lactobacillus plantarum</i> 7. <i>Bifidobacterium longum</i> 8. <i>Akkermansia muciniphila</i> 9. <i>Pseudomonas aeruginosa</i>	<a href="https://github.com/euba/BacArena">https://github.com/euba/BacArena</a>	Bauer et al. [30].
MCM	UNIX/ LINUX	A dynamical framework for modeling microbial communities, which combines genome scale metabolic reconstructions with environmental variables and arbitrary reaction kinetics	<i>E. coli</i>	<a href="http://www.zoology.ubc.ca/MCM">http://www.zoology.ubc.ca/MCM</a>	Louca and Doebeli [29].
COMETS	UNIX/ LINUX	A multi-scale modeling framework that integrates spatiotemporal dynamics of microbial community with stoichiometric models	1. <i>E. coli</i> 2. <i>Salmonella enterica</i> 3. <i>Methylobacterium extorquens</i>	<a href="https://github.com/segrelab/comets">https://github.com/segrelab/comets</a>	Harcombe et al. [28].
d-optCom	UNIX/ LINUX	A multi-level and multi-objective simulation of microbial communities that incorporates the dynamic information of biomass concentrations and shared metabolites	1. <i>E. coli</i> 2. <i>Geobacter sulfurreducens</i> 3. <i>Rhodospirillum rubrum</i> 4. <i>Shewanella oneidensis</i>	–	Zomorodi et al. [27].
DyMMM	MATLAB	A pioneer dynamic framework to integrate GEMs (add-on to the COBRA toolbox)	1. <i>Geobacter sulfurreducens</i> 2. <i>Rhodospirillum rubrum</i>	<a href="https://sourceforge.net/projects/dymmm/">https://sourceforge.net/projects/dymmm/</a>	Zhouang et al. [26].

the effect of the microbiota on host metabolism and highlighted that the gut microbiome leads to a depletion of host glutathione synthesis, in addition to regulating host amino acid and lipid metabolism [45].

### 3.2. Metabolic conditions and microbiota

The advent of omics technologies enabled the identification of key traits of microbiota associated with diet and obesity. Combined analysis of multiple omics data from a longitudinal weight perturbation study revealed that weight changes had extensive molecular signatures of chronic diseases such as hypertrophic cardiomyopathy and insulin resistance [46]. The pathophysiologic role of adipose tissue on the progression of obesity is well established; white adipose tissue can be a rational target to prevent obesity and related disorders. The generation of GEMs for adipocytes and the integration of gene expression and plasma metabolomics data onto the model enabled mechanistic explanations of the metabolic differences between lean and obese individuals. Model predictions (e.g. decreased glutaminolysis and alterations in the glutamate, pyruvate, and  $\alpha$ -ketoglutarate metabolism) were consistent with the results from human subjects [47]. Moreover, a recent study revealed that energy-dense diets altered the diversity of the microbial composition and increased mucosa permeability of the ileum and colon in obese mice with NAFLD. The authors also found that plasma SCFA levels were correlated with specific groups of bacteria, and specific bacterial taxa associated with disease-associated factors were also positively correlated with isoacid SCFAs. In addition, dietary fermentable fibers were found to alter microbiota-derived signals and regulate the gene expression and metabolic

pathways of the liver by reducing host nitrogen and amino acid homeostasis [48].

Diabetes mellitus (DM) is a major public health concern, affecting over 425 million people worldwide [49]. Increasing evidence indicates a contributing role of gut microbiome in the pathophysiology of DM. An integrative taxonomic and functional analysis of multiple meta-omics data revealed that intra- and inter-individual variation of microbiota composition in the context of type 1 DM were strongly affected by family membership [50]. Similarly, two large cohorts of healthy individuals showed that gut microbiome composition was more affected by environmental influences (e.g. household sharing, diet, lifestyle) instead of host genetics [51]. Several predictive metagenomic tools described an association with obesity, insulin resistance and T2DM. Gut microbiome 16S rRNA sequencing and metagenomics profiling of T2DM zebrafish model revealed similar features with human T2DM, such as lower taxa richness, higher faecal and plasma fructose and BCAA concentrations, reduced fecal butyrate levels, and altered pathways of amino acid and sugar metabolism [52].

### 3.3. Drug metabolism and microbiota

The bidirectional interaction of gut microbiota and drug metabolism have been reported for more than 50 pharmaceuticals [53]. Gut microbiome composition has recently been associated with the efficacy and toxicity profiles of commonly used drugs, but the extent of this relationship remains largely unknown. Drug-microbe networks serve as a novel step towards revealing the role of the microbiome in drug metabolism. For instance, Zimmerman et al. identified 30 human gut microbiome-encoded



enzymes responsible for the biotransformation of 20 drugs to 59 candidate metabolites, which suggests that drug-metabolizing activities of human gut microbiota may differ on the basis of interpersonal variation of microbial genomic contents [54]. In another comprehensive study, screening of more than 1000 marketed non-antibiotic drugs against the growth of gut bacterial strains revealed that 24% of the drugs showed antibiotic-like effects and the inhibition of bacterial growth was strongly correlated between non-antibiotic and antibiotic drugs, which implies antibiotic resistance on the basis of gut microbiome composition, after regular consumption of non-antibiotic drugs [55]. Similarly, a recent study proposed an interplay between decreased *Bacteroides fragilis* increased glyoursodeoxycholic acid – inhibition of intestinal FXR signaling; which might explain how metformin improves hyperglycemia. Hence, this study identified glyoursodeoxycholic acid as a potential target for the treatment of T2D [56]. The findings of this study are concordant with accumulating evidence that associates the antihyperglycemic effect of metformin being modulated by gut microbiota, which was recently proven in double-blind metagenomics and targeted metabolomics research [57].

#### 4. Role of microbiota in hepatic diseases

In the context that the enterohepatic circulation links the gut microbiota and liver through the transport of the intermediate end-products of microbial metabolism, efforts to uncover the potential physiological impact of the gut microbiome on liver damage have lately been increased. In recent years, several lines of human and animal research indicated that the gut microbiome represented a significant environmental factor that contributed to the development of several liver diseases and its progression into end-stage cirrhosis, as well as cancer [58,59]. In addition to prompting the use of microbiome-based approaches for the diagnosis of liver diseases, evidence also shows that this may be a primary target for the treatment of diseases. Taking advantage of the individual gut microbiota makeup, which can be manipulated via a range of methods, selection of suitable fecal mass transplant donors and designation of potential effective pro- and/or prebiotics can be achieved so as to alleviate the pathologies as well as conceivably devise personalized therapies for various liver diseases.

##### 4.1. Non-alcoholic fatty liver disease

NAFLD has been defined as global burden because it is the leading cause of chronic liver disease and affects around 25% of the global population [60]. The pathogenesis of NAFLD begins with the simple accumulation of lipid in hepatocytes and progresses to hepatocellular damage and inflammation (non-alcoholic steatohepatitis, NASH), which can progress to cirrhosis [61]. The pathophysiology of NAFLD has not yet been elucidated, but several human and animal studies have confirmed the contribution of intestinal microbiota as a driver through the compositional changes, altered microbiota-derived metabolites, and impaired gut-barrier integrity [62–64]. A definite understanding of the molecular basis of the gut and liver bidirectional relation is a prerequisite for developing non-invasive approaches with a robust discriminative ability for determining the presence and severity of NAFLD, as well as treating NAFLD and NASH by the use of precision pharmacotherapies.

Despite the common use of advanced fibrosis as the primary determinant in predicting liver destruction, its rigorous detection by way of using markers associated with gut microbiota lacks extensive and concordant data. For this aim, a study in a biopsy-proven population of adult patients with NAFLD evaluated the association between gut dysbiosis and the severity of NAFLD lesions by using 16S rRNA gene sequencing of stool samples. Multivariate analysis of the results indicated that *Bacteroides* abundance and

*Prevotella* depletion were observed in people with NASH and *Ruminococcus* abundance was associated with fibrosis [65]. Likewise, accurate stage prediction of liver disease (from mild/moderate NAFLD to advanced fibrosis) was characterized using a panel of intestinal microbiota-derived signatures. Metagenomics of stool microbiome and serum metabolome analysis data were used to build a random forest classifier model with a set of 40 features, with which advanced fibrosis was distinguished by an increased abundance of Proteobacteria and *E. coli* and a decreased abundance in Firmicutes [66]. Another multiomics study that claims specific gut microbiota states signal to pathologic conditions in differing stages is supported with a metagenomics and metabolomics study of pediatric patients with NAFLD. The findings suggested that a decrease in *Oscillospira* coupled with 2-butanone enrichment was found to be a microbiome signature for NAFLD onset and *Ruminococcus*, *Blautia*, and *Dorea* were significantly increased with NASH progression [67].

Integration of high-quality genome-scale metabolic models with multi-omics data of patients has been of considerable interest in exploring diet-microbiota interactions to understand the pathogenesis and prevention of NAFLD. In a recent study, authors showed the dramatic benefits of an isocaloric low-carbohydrate/increased protein diet in obese subjects with NAFLD. The researchers performed in-depth multi-omics profiling (included plasma metabolomics and liver transcriptomics) after a short-term dietary intervention and combined the data with a genome-scale metabolic model of hepatocytes. They observed rapid and marked reductions in hepatic de novo lipogenesis, augmented serum b-hydroxybutyrate concentrations compatible with mitochondrial beta-oxidation, and significant microbial changes toward folate-producing *Streptococcus* resulting in increased serum folate concentrations. Overall, the results indicated that carbohydrate-restricted diets shaped the gut microbiome composition and held potential for the treatment of NAFLD [68]. Another comprehensive study investigated the underlying metabolic differences in NAFLD using systems-level approaches coupled with mice experiments and a proof-of-concept human study. The investigators integrated the metabolomic measurements of each subject with a liver GEM to simulate individual liver metabolism. Their analysis revealed that plasma levels of glycine, serine, betaine, and N-acetyl-glycine (precursors for glutathione and NAD<sup>+</sup> biosynthesis) were negatively correlated in subjects with high degrees of hepatic steatosis, and dietary serine supplementation was likely an effective treatment strategy [69].

##### 4.2. Alcoholic liver disease

ALD is damage to the liver caused by excess alcohol intake. The spectrum of disease ranges from fatty liver, to hepatitis and cirrhosis. Misuse of alcohol affects the composition and function of gut microbiota, which could initiate or potentiate liver disease [70]. Several animal studies indicated that composition of gut microbiota might be responsible for the consequences of ALD including raised liver inflammation, weakened immune system, and alterations in microbial metabolism products [71–73]. Correspondingly, shotgun metagenomes of patients with alcohol dependence and liver dysfunction were associated with depletion of many commensal gut taxa and community shifts, including the reduction of multiple *Clostridiales* and *Bacteroidales* members, but enrichment in *Bifidobacterium* and *Lactobacillus* and oral-origin microbiota. Molecular changes in the course of ALD encompass bacterial overgrowth due to sparsity of the geni *Blautia*, *Lachnospiraceae*, *Faecalibacterium* and *Roseburia*, and raised serum concentrations of endotoxins that leak from the gut wall in the presence of acetaldehyde [74].

Computational modeling and manipulation of the ALD network provides the means to form sound estimations regarding the chief



inflammatory cascades and other molecular interactions active in this disease. For instance, interactome and transcriptome data of ALD were used to reconstruct static and dynamic networks that were consistent with the important role of key signaling pathways (TLR4, NF- $\kappa$ B, MAPK and apoptosis) in ALD. The findings of this study allow for the emergence of varied classes of data, enabling the representation of molecular networks and signaling cascades, and thus paving the way for new drugs targeting ALD [75].

#### 4.3. Liver cirrhosis

Cirrhosis is scarring of the hepatic tissue, mainly seen at the late stages of chronic liver disease. Alterations of gut microbiota composition and function is significant in liver cirrhosis, but the underlying mechanisms are still unresolved. Disruption of the intestinal barrier by the altered gut microbiome and systemic inflammation with bacterial products are the proposed key players in the advancement and related complications of liver cirrhosis [76].

Combined metagenomic and metabolic assays could provide significant advantages to decipher the physiology of gut microbiome in cirrhosis states. For instance, a comparison of fecal microbiota of patients with cirrhosis and controls revealed a decrease in Bacteroidetes and enrichment in *Enterobacteriaceae* and *Veillonella*. This functional diversity resulted in an enrichment in the metabolism of toxins depredation and nutrient absorption, but also a depletion of bile acid and cell cycle-related metabolism [77]. Likewise, a recent meta-omics-based study directly linked microbial dynamics to liver cirrhosis through metabolites by reporting a significant decrease of bacteria involved in the digestion of non-starch polysaccharides (e.g. *Alistipes* sp.HG5, *Clostridium thermocellum*) and butyrate-producing bacteria, and a marked increase of opportunistic pathogens during disease progression. This impaired homeostasis of the gut may be responsible for the disorganized intestinal barrier, which allows endotoxins and pathogens into the hepatic circulation, resulting in systemic inflammation [78].

Systems biology approaches of human gut microbiota are promising tools for the estimation of disease progression. For instance, Bajaj et al. described a quantitative index of dysbiosis accompanying cirrhosis severity in a comprehensive, well-characterized population within a range of individuals from healthy controls to those with end-stage cirrhosis. The cirrhosis dysbiosis ratio reflects changes of autochthonous to non-autochthonous taxa such as the negative correlation with disease progression as well as endotoxemia; however, the ratio was stable if disease remained unchanged, which may be useful in clinical practice to evaluate changes in microbiome accompanying cirrhosis progression [79].

#### 4.4. Hepatocellular carcinoma

HCC is the most common primary malignancy of the liver. Accumulating evidence suggests that the gut microbiota has been involved in the pathogenesis of HCC. Advancement of chronic liver disease to HCC has been linked to inflammatory pathways, which are activated by the disruption of intestinal mucosa and translocation of endotoxins to the portal veins as a result of imbalanced gut microbiota [80]. As novel evidence, a correlation model of the features (such as microbiota profile, intestinal permeability, inflammatory status, and circulating mononuclear cells), which possibly link microbiota with HCC, demonstrated that *Enterococaceae*, *Streptococcus*, *Bacteroides*, and *Ruminococcaceae* were increased in patients with HCC, whereas *Akkermansia* and *Bifidobacterium* were reduced. Plasma levels of inflammatory markers (IL8, IL13, CCL3, CCL4, and CCL5) and circulating monocytes were higher in the HCC group. These findings propose that replacement of mucosa protective bacteria with LPS-producing bacteria is associated with intestinal and

systemic inflammation and may promote the development of HCC [81].

By reason of symptoms being nonspecific in initial stages, there is an urgent need for novel diagnostic and therapeutic targets for HCC. Alterations of gut microbiota will likely provide a potential biomarker for the prediction of early stage HCC. A systematic investigation of microbiota in early HCC across a large clinical cohort from three different regions of China demonstrated a depletion of *Akkermansia* and butyrate-producing bacteria (e.g. *Ruminococcus*, *Oscillibacter*, *Faecalibacterium*, *Clostridium IV*, and *Coproccoccus*), and enrichment of gram-negative species (e.g. *Klebsiella* and *Haemophilus*). The authors proposed 30 biomarkers identified by random forest models that might be used as non-invasive diagnostic tools of HCC through further validation of these results in different cohorts from different countries and ethnicities [82].

### 5. Conclusion and outstanding questions

The interactions between the gut microbiome and human host metabolism has received increasing attention in the context of understanding the potential role of the gut microbiome in health and various complex conditions including obesity, DM and liver diseases. Regardless of the fact that increasing evidence from numerous studies suggest that gut microbiota contributes to overall health and disease, one should be careful whilst interpreting the results; (1) the strict description of a healthy microbiota is still obscure due to the taxonomic composition and the definitive interactions between gut microorganisms shared among healthy individuals are not yet well-characterized; (2) considering the complex ecosystem of the microbiome is ever-changing, determination of the relevant measures of functional capacity and stability are affected by multiple factors including environmental and host-related influences; (3) the genes in the microbiome were mapped to their functions; however, the genetic information may not always be reflected in the phenotype or may not perform the expected function; (4) although the taxonomic profile of the human gut microbiome is not affected in the long term, the dynamic structure of the microbiota makes it difficult to predict the outcome of dietary interventions, and the long-term results of these interventions are unknown; (5) the species-level findings in the gut microbiota research are not consistent, which may be a consequence of the study-related discrepancies, such as patient cohorts, comparison groups, definition of disease; (6) the reproducibility of results is low and may result in bias in the interpretation of results because sample collection, sequencing, and analysis of high-throughput methodologies are not standardized; (7) systems biology methodologies have proven to be a valuable tool in individual cells and tissues, but the computational models for bacteria and the algorithms developed for understanding microbial communities are still in their infancy. Nevertheless, through the advancement in high throughput technologies and methods that are used in system biology-based studies, the effect of the gut microbiome on human health may be better understood, which could lead to the development of preventive and personalized treatment strategies.

#### Search strategy and selection criteria

Data for this review were identified by searches of PubMed, Google Scholar, and references from relevant articles using the search terms “microbiome”, “microbiota”, “liver”, “omics”, “biomarker”, “personalized medicine”, and “genome-scale metabolic model”. Only articles published in English between 2010 and 2019 were included, with a preference for those published after 2016.

**Box 1. Omics technologies in microbiome studies.****Metagenomics**

Metagenomic studies have already revealed novel insights into the diversity, population structure and dynamics of microbiota composition [83]. The link between the functional genes of gut microorganisms and the pathogenesis of metabolic diseases, such as diabetes and obesity, are already well established [84]. Integrating omics data with biologic networks has been used in the identification of biomarkers for the development of detection and treatment strategies [85]. Although metagenomics provides useful tools for evidence-based studies, these approaches have bottlenecks in terms of high-quality annotation, assignment of functional information into uncharacterized community structures, and interpretation of comparative investigations, regardless of environmental conditions [86].

**Metatranscriptomics**

Actively expressed genes of the human gut microbiome enable an understanding of the potential functions of a microbial community and dynamic interactions with the host [87]. Next-generation sequencing technologies enable the identification of mRNA expression profiles, including novel non-coding RNAs such as small RNAs associated with central biologic processes [88]. The main limitation of this approach is the difficulty in the detection of bacterial mRNAs due to their short half-life on the order of minutes. In addition, assembly of non-continuous short-read sequences and repeated patterns renders the process even more problematic [89].

**Metaproteomics**

The determination of a complete profile of gene translation products expressed within a microbiome and their post-translational alterations is necessary to elucidate the species involved in specific functions by assigning proteins to specific taxa [90]. Metaproteomics data integrated with computational workflows have been used to decipher active pathways in a microbial composition to explore the complex interactions of the human gut microbiome and the host [91]. As a result, metaproteomics provides new knowledge not garnered by metagenomics; however, standardized metaproteomics protocols are needed to compare studies and relate protein abundances to microbial functions [92].

**Metabolomics**

Analysis of the biochemical profiles of metabolism simultaneously implies the current physiology of microbial activities [93]. Measuring the compositions and concentrations of low-molecular-weight compounds in a state of flux is commonly used in the discovery of potential biomarkers and drug targets [94]. The identification of metabolite production is complicated in the context of mixed microbial communities, thus the combination of other omics technologies and stable isotope probing techniques with metabolomics has become highly useful [95].

**Declaration of Competing Interest**

The authors declare no conflict of interest.

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